preparation of *E. coli* DAGK in lysophospholipids, LMPC and LMPG, allows the protein to be catalytically active as observed using a spectrophotometric assay. We also found that the ^{15}N-TROSY-HSQC spectrum quality of these samples is comparable to that of a sample prepared in dodecylphosphocholine (DPC), which is the detergent used to determine the structure of *E. coli* DAGK in the absence of the substrates. In addition, we noticed that in the two lysophospholipid conditions, even though addition of substrates does not alter peak dispersion on the ^{15}N-TROSY-HSQC spectrum of DAGK significantly, we can map the catalytic site by monitoring the peaks that shifts as the substrates are titrated. Altogether, our data indicate that the use of lysophospholipids in sample preparation allows us to acquire structural information of DAGK in its active conformation with the substrates bound at the catalytic site. This work is supported by NIH grant R01 GM47485.

1004-Plat
Mixing and Matching Detergents for Membrane Protein NMR Structure Determination
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One major obstacle to membrane protein structure determination is the selection of a detergent micelle that mimics the native lipid bilayer. Currently, detergents are selected by exhaustive screening because the effects of protein-detergent interactions on protein structure are poorly understood. In this study, the structure and dynamics of an integral membrane protein in different detergents is investigated by nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) spectroscopy, and small angle X-ray scattering (SAXS). The results suggest that matching of the micelle dimensions to the protein's hydrophobic surface avoids exchange processes that reduce the completeness of the NMR observations. Based on these dimensions, several mixed micelles were designed that improved the completeness of NMR observations. These findings provide a basis for the rational design of mixed micelles that may advance membrane protein structure determination by NMR.

1005-Plat
Structure and Dynamics of TM Domains of Human Glycine Receptor in LPPG Micelles
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The structure and backbone dynamics of the entire four transmembrane domains of human glycine receptor α1 subunit (GlyRTM1234) were studied in LPPG micelle. Using triple-resonance multi-dimensional NMR methods, over 80% residues were assigned. The chemical shift index clearly showed that most residues in the TM region were in helical conformation, consistent with a four-helix-bundle TM protein. We have collected sufficient amount of distance, H-bond, and dihedral angle restraints from NOE and chemical shift data, as well as long-range distance restraints from paramagnetic relaxation enhancement measurements. The structure model, determined using the available restraints, showed a kink between Trp72 and Cys76 in the beginning of TM3 domain. Moreover, the end of TM2 domain, S53 to S56, is more flexible compared to the rest of TM2. In contrast, part of TM23 loop, V63 to V66, showed a helical secondary structure. Backbone dynamics measurements indicated the existence of significant internal motions of TM1234 in LPPG micelles. The relaxation data also resulted in an overall rotational correlation time of ~33.4 ns, estimated based on the non-flexible helical residues. This overall tumbling time corresponds to a 66.8 kDa protein-LPPG complex with ~98 LPPG molecules per GlyRTM1234. The size is confirmed by the dynamic light scattering measurement. We suggest that the flexible end of TM2 and the beginning of TM3 can better coordinate the coupling between the TM2-TM3 loop and the extracellular domain. This coupling is thought to be important for mediating channel gating. In addition, it is confirmed that the early termination of TM2 helix in GlyR is an intrinsic property and is independent of the presence or absence of other TM domains (Funded by NIH R37GM049202 & R01GM069766).

1006-Plat
Conformational Cycle Of A Bacterial Homolog Of Human Neurotransmitter Sodium Symporters
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Neurotransmitter:sodium symporters (NSS) control the magnitude and duration of synaptic signaling through active reuptake of specific neurotransmitters. While it is known that the transmembrane sodium gradient supplies the energy for transport, the coupling mechanism to substrate translocation from its primary binding site (S1) is poorly understood. Conformational rearrangements of extracellular and cytoplasmic regions of the protein are thought to regulate alternating access to S1, and substrate binding at a second site (S2) located near the extracellular region of the transporter, to act as a symport coupler (Shi et al 2008 Mol Cell 30, 667). We used spin labeling and EPR spectroscopy to investigate the conformational dynamics of a highly homologous bacterial member of the NSS family, the leucine transporter LeuT, for which the crystal structure is known (Yamashita et al 2005 *Nature* 437, 215). Changes in global and local structural constraints derived from the EPR analysis and induced by sodium and leucine binding were then correlated to conformational changes in the LeuT structure in proteoliposomes. Sodium binding was found to increase the distance between the probes as assessed from global rearrangements measured in the extracellular region of LeuT. In contrast, subsequent leucine binding in the presence of sodium was found to decrease the distance between the probes. Consistent with these observations, sodium binding increases spin label mobility and water accessibility at positions within the S2 site. Furthermore, leucine binding increases spin label order and decreases water accessibility. These results suggest a model in which sodium binding to LeuT primes the transporter for substrate binding in S1 by increasing the population of an "outward-facing" conformation, exposing the substrate permeation pathway. With a filled S1, the extracellular pathway constricts, consistent with the occluded state observed in the LeuT crystal structure.

1007-Plat
X-ray Footprinting Studies on Photoactivation of Bovine Rhodopsin
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Rhodopsin, the visual G-protein coupled receptor (GPCR) in the rod cells of the vertebrate retina, is fundamental to vision. The light-activated intermediate of rhodopsin, Meta-II, initiates a signalling cascade that culminates in an electrical impulse in the visual cortex of the brain. The molecular details of agonist-induced structural change that are likely to be conserved among the members of the GPCR super family are not fully understood. We used X-ray Footprinting to study the conformational change in rhodopsin in solution upon photoactivation. Purified rhodopsin is exposed in tens of milliseconds with high flux focused X-rays. The hydroxyl radicals that are generated by photolysis of water react with the solvent accessible side chains and form stable modification products. The peptic fragments are analyzed by mass spectrometry to quantify the extent and identify the sites of oxidation. Monitoring the changes in the radiolytic modification as function of the exposure time provides information that is directly correlated with the solvent accessibilities of individual peptide or side chain residues within the protein. The difference in solvent accessibilities between dark state and light activated Meta-II state shows conformational changes near the retinal binding site, but not a large structural change as predicted by some models of GPCR activation. Labeling was also observed in the trans-membrane helical regions, this was also unexpected. We introduce a novel O^{18} labeling method to determine if transmembrane labeling arises from exchange with bulk water or is mediated by activation of bound, conserved water molecules in the GPCR structure. This is a novel approach that can probe the details of bound water structure and dynamics that function in a number of ion channels and receptors.

Platform M: Cell & Bacterial Mechanics Motility

1008-Plat
Physical Description of Mitotic Spindle Orientation During Cell Division
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During cell division, the duplicated chromosomes are physically separated by the action of the mitotic spindle. The mitotic spindle is a dynamic structure of the cytoskeleton, which consists of two microtubule asters. Its orientation defines the axis along which the cell divides. Recent experiments on dividing cells, which adhere to patterned substrates, show that the spindle orientation depends on the spatial distribution of cell adhesion sites. In the present work we show that